Express Mail Label No. EL 405 598 025 US

03-13-00 A

jc784 U.S. PTO 03/10/00

UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. 0152.00355

Total Pages in this Submission

jc525 U.S. PTO 09/523055 03/10/00

TO THE ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

INTERRUPTING THE INTERACTION OF INTERCELLULAR ADHESION MOLECULE-1 AND RESPIRATORY SYNCYTIAL VIRUS TO FOR PREVENTION AND TREATMENT OF INFECTION

and invented by:

Aruna K. Behera; Hiroto Matsuse; Mukesh Kumar; Hamid Rabb; Richard F. Lockey; Shyam S. Mohapatra

If a CONTINUATION APPLICATION, *check appropriate box and supply the requisite information:*

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Which is a:

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Which is a:

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Enclosed are:

Application Elements

☐ 1. ☒ Filing fee as calculated and transmitted as described below

☐ 2. ☒ Specification having 33 pages and including the following:

a. ☒ Descriptive Title of the Invention

b. ☒ Cross References to Related Applications *(if applicable)*

c. ☐ Statement Regarding Federally-sponsored Research/Development *(if applicable)*

d. ☐ Reference to Microfiche Appendix *(if applicable)*

e. ☒ Background of the Invention

f. ☒ Brief Summary of the Invention

g. ☒ Brief Description of the Drawings *(if drawings filed)*

h. ☒ Detailed Description

i. ☒ Claim(s) as Classified Below

j. ☒ Abstract of the Disclosure

EL405598025US

March 10, 2000

37 CFR ... Washington, D.C.

Constance McLean
(Signature of person mailing paper or fee)

UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. 0152.00355
Total Pages in this Submission

Application Elements (Continued)

3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*

   a. ☐ Formal b. ☒ Informal Number of Sheets 4

4. ☒ Oath or Declaration

   a. ☐ Newly executed *(original or copy)* ☒ Unexecuted

   b. ☐ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*

   c. ☒ With Power of Attorney ☐ Without Power of Attorney

   d. ☐ *DELETION OF INVENTOR(S)*
   Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. 1.63(d)(2) and 1.33(b).

5. ☐ Incorporation By Reference *(usable if Box 4b is checked)*
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Computer Program in Microfiche

7. ☐ Genetic Sequence Submission *(if applicable, all must be included)*

   a. ☐ Paper Copy

   b. ☐ Computer Readable Copy

   c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☐ Assignment Papers *(cover sheet & documents)*

9. ☐ 37 CFR 3.73(b) Statement *(when there is an assignee)*

10. ☐ English Translation Document *(if applicable)*

11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations

12. ☐ Preliminary Amendment

13. ☒ Acknowledgment postcard

14. ☒ Certificate of Mailing

    ☐ First Class ☒ Express Mail *(Specify Label No.):* EL 405 598 025 US

UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
0152.00355
Total Pages in this Submission

Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*

16. ☒ Small Entity Statement(s) - Specify Number of Statements Submitted: 2

17. ☐ Additional Enclosures *(please identify below):*

Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	18	- 20 =	0	x $9.00	$0.00
Indep. Claims	7	- 3 =	4	x $39.00	$156.00
Multiple Dependent Claims (check if applicable) ☐					$0.00
				BASIC FEE	$345.00
OTHER FEE *(specify purpose)*					$0.00
				TOTAL FILING FEE	$501.00

☒ A check in the amount of **$501.00** to cover the filing fee is enclosed.

☒ The Commissioner is hereby authorized to charge and credit Deposit Account No. **11-1449** as described below. A duplicate copy of this sheet is enclosed.

- ☐ Charge the amount of as filing fee.
- ☒ Credit any overpayment.
- ☒ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Dated: **March 10, 2000**

Signature

Kenneth I. Kohn, Reg. No. 30,955
KOHN & ASSOCIATES
30500 Northwestern Highway
Suite 410
Farmington Hills, Michigan 48334
(248) 539-5050
FAX: (248) 539-5055

cc:

PATENT

Attorney's Docket Number: 0152.00355

Applicant or Patentee: Aruna K. Behera

Serial or Patent No: _____

Filed or Issued: _____

Title: INTERRUPTING THE INTERACTION OF INTERCELLULAR ADHESION MOLECULE-1 AND RESPIRATORY SYNCYTIAL VIRUS TO FOR PREVENTION AND TREATMENT OF INFECTION

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS
[37 CFR 1.9(f) and 1.27(c)]-SMALL BUSINESS CONCERN

I hereby declare that I am:

_____ the owner of the small business concern identified below:

__X__ an official of the small business concern empowered to act on behalf of the concern identified below:

Name of Concern: University of South Florida Research Foundation, Inc.

Address of Concern: 4202 East Fowler Avenue - FAO 126

Tampa, Florida 33620-4962

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement: (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when, either directly or indirectly, one concern controls or has the power to control the other, or a third-party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention described in:

__X__ the specification filed herewith with title as listed above.

_____ the application identified above.

_____ the patent identified above.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c), if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

*** NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)**

Each such person, concern or organization having any rights in the invention is listed below:

_____ No such person, concern, or organization exists.

__X__ Each such person, concern or organization is listed below.

NAME: University of South Florida

ADDRESS: 4202 East Fowler Avenue - FAO 126

Tampa, Florida 33620-4962

___ Individual ___ Small Business __x__ Nonprofit Organization

NAME: _____

ADDRESS: _____

_____

___ Individual ___ Small Business ___ Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28(b)]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: Kenneth G. Preston

Title in Organization: Executive Director

Address of Person Signing: 4202 East Fowler Avenue - FAO 126

Tampa, Florida 33620-4962

SIGNATURE: _____ **Date:** 3-10-00

Attorney's Docket Number: 0152.00355

Applicant or Patentee: Aruna K. Behera et al.

Serial or Patent No: _____

Filed or Issued: _____

Title: INTERRUPTING THE INTERACTION OF INTERCELLULAR ADHESION MOLECULE-1 AND RESPIRATORY SYNCYTIAL VIRUS TO FOR PREVENTION AND TREATMENT OF INFECTION

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(d)--NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

Name of Organization: University of South Florida -

Organization Address: 4202 East Fowler Avenue - FAO 126

Tampa, Florida 33620-4962

Type of Organization:

_X_ University or other Institution of Higher Education

___ Tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3)

___ Nonprofit Scientific or Educational under Statute of State of the United States of America State: _____ Citation of Statute: _____

___ Would Qualify as Tax Exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3) if located in the United States of America State: _____ Citation of Statute: _____

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under Section 41(a) and 41(b) of Title 35, of United States Code with regard to the invention described in:

_X_ the specification filed herewith with title listed above.

___ the application identified above.

___ the patent identified above.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or nonprofit organization under 37 CFR 1.9(e).

* NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME: University of South Florida Research Foundation, Inc.

ADDRESS: 4202 East Fowler Avenue - FAO 126

Tampa, Florida 33620-4962

___Individual _X_Small Business ___Nonprofit Organization

NAME: _____

ADDRESS: _____

_____

___Individual ___Small Business ___Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28(b)]

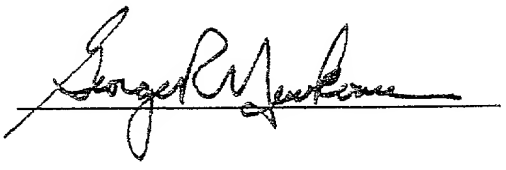
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: George R. Newkome, Ph.D.

Title in Organization: Vice President for Research

Address of Person Signing: 4202 East Fowler Avenue - FAO 126

Tampa, Florida 33620-4962

SIGNATURE:  Date. 3-10-00

0152.00355

INTERRUPTING THE INTERACTION OF INTERCELLULAR ADHESION MOLECULE-1 AND RESPIRATORY SYNCYTIAL VIRUS TO FOR PREVENTION AND TREATMENT OF INFECTION

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a conversion of U.S. Provisional Application, Serial No. 60/123,999 filed March 11, 1999, which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

The present invention relates to a method and compound for prevention and/or treatment of respiratory viral infection. More specifically, the present invention relates to blocking Respiratory Syncytial Virus binding to ICAM-1 via agents that interfere with binding or block the expression of ICAM-1

2. DESCRIPTION OF RELATED ART

Respiratory viruses such as respiratory syncytial virus (RSV), the parainfluenza viruses (PIV), and the influenza viruses cause severe lower respiratory tract diseases in infants and children throughout the world. It is also an important cause of disease in adults and is responsible for a significant amount of excess morbidity and mortality in the elderly. It also can be devastating in immunosuppressed populations (Murray et al., 1997; Pullen et al. 1982; Hall et al. 1984).

Experimental live attenuated vaccines for each of these viruses are being developed for intranasal administration in the first weeks or months of life, but none are currently FDA approved. A variety of RSV, PIV-3, and influenza virus vaccine strains have been developed by classical biological methods, evaluated extensively in preclinical and clinical studies, and shown to be attenuated and genetically stable. However, a major remaining obstacle to successful immunization of infants against respiratory virus associated disease may be the

relatively poor immune response of very young infants to primary virus infection. (Crowe JE Jr Vaccine 1998 Aug-Sep;16(14-15):1423-32 Immune responses of infants to infection with respiratory viruses and live attenuated respiratory virus candidate vaccines.)

Moreover, even if one or more vaccines are approved, they may not be suitable for some populations vulnerable to RSV (e.g. very young infants and the immunosuppressed). Ribavirin and immunoglobulin preparations with high titers of RSV-specific neutralizing antibodies are currently approved for use to treat and prevent RSV infection. However, neither of these methods are cost-effective or simple to administer. New agents are needed to reduce the impact of RSV. (Wyde PR Antiviral Res 1998 Aug;39(2):63-79 Respiratory syncytial virus (RSV) disease and prospects for its control.

Data obtained from the National Respiratory and Enteric Virus Surveillance System demonstrates the seasonal pattern of RSV infection, with peak rates of 30-40% occurring at the beginning of each year (Murray et al., 1997; Pullen et al. 1982; Hall et al., 1984). RSV infection is commonly associated with interstitial lung diseases, such as bronchiolitis and asthma. It is a major risk factor for a number of other disease conditions, such as immunodeficiency, cardiac arrhythmia, congenital heart disease, and unusual atrial tachycardia (Sly, et al., 1989; Robinson et al. 1997; Armstrong et al. 1993; Fixler, 1996; Lemen, 1995; Persson, 1997; Shelhamer et al. 1995).

Although the severity of the disease decreases with repeated infection, previous RSV infection renders no or limited immunity to subsequent RSV infection (Hal, 1991).

Despite the above serious implications of RSV infection, the progress in the knowledge of the viral genes and gene products (Collins, 1991; Collins et al., 1996; Barik, 1992), an effective vaccine, or treatment against RSV, is yet to be developed.

Additionally, previous attempts to develop a vaccine using formalin inactivated RSV not only failed but exacerbated the disease when subsequent RSV infection occurred (Chanock, et al. 1992; Hall, 1994). An effective vaccine or treatment for RSV would be highly desirable.

Additionally, human nasal, airway, and lung epithelial cells constitute a major target for respiratory infections. Viral infection alters the expression of genes encoding a number of cytokines, chemokines and inflammatory mediators (Sabauste, et al. 1995; Choi, et al. 1992; Becker et al. 1993

The secretion of cytokines by airway epithelial cells can either initiate local inflammatory responses or amplify an inflammatory event that was previously initiated by activated macrophages, eosinophils, mast cells or lymphocytes (Shelhamer et al., 1995; Holtzman, et al. 1991; Churchill, et al. 1989; Marini, et al. 1992; Churchill, et al. 1992; Kwon, et al. 1994; Sousa, et al. 1994; Cromwell, et al. 1992; Jin, et al. 1997). The epithelial cell-mediated inflammation by involve a number of cytokines and chemokines including IL-1β, IL-6, IL-8, IL-11, IFN-γ, TNF-α, GM-CSF, GRO-α, PLA-2, C3, inducible nitric oxide synthase (iNOS), MCP-1, endothelin-1 (ET-1), mucin, elastase-specific inhibitors, and secretory leukocyte proteinase inhibitor.

The rhinovirus infection of a transformed HBE cell line, BEAS-2B, caused the release of the granulocyte macrophage colony stimulating factor (GM-CSF), IL-6, and IL-8 (Sabauste et al. 1995). The influenza virus infection of primary cultures of human bronchial epithelial (HBE) cells induced the expression of IL-8 (Choi, et al. 1992). Also, in response to RSV infection, nasal epithelial cells and BEAS-2B cells generated IL-8 (Becker et al., 1993; Merolla et al., 1995; Noah, et al., 1993; Garofalo et al. 1996).

It is known that, when infected bronchial epithelial cells secrete several pro-inflammatory cytokines, as set forth above. Some of these cytokines (IL-1β, TNF-2) up-regulate ICAM-1 expression on these cells (Persson et al., 1997; Becker et al., 1993; Noah et al., 1993; Sabauste et al., 1995). ICAM-1, a member of the immunoglobulin gene super family, is a cell surface receptor for

the lymphocyte function-associated antigen (LFA-1) adhesion molecule (Makgoba et al., 1998). ICAM-1 mediates the integration of leukocytes into inflammatory sites and facilitates interaction between lymphocytes and target cells. ICAM-1 is also the major cell surface receptor for many of the rhinoviruses (Greve et al., 1989; Staunton et al., 1989; Tomassini et al., 1989).

RSV, though phylogenetically different from rhinovirus, induces a similar profile of cytokines in epithelial cells and also NF-κB, which regulates expression of ICAM-1 that plays a role in neutrophil and eosinophil adhesion to epithelial cells (Arnold et al., 1995; Chini et al., 1998; Stark et al., 1996). Also an elevated expression of ICAM-1 in nasal epithelial cells of asthmatics has been reported (Vignola et al., 1993).

SUMMARY OF THE INVENTION

According to the present invention, there is provided a method of preventing a respiratory infection by administering an effective amount of an agent for regulating ICAM-1 expression. Also provided is a composition for the prevention of respiratory infection including an agent which regulates ICAM expression. method of preventing RSV infection by administering an effective amount of an agent that interferes with the binding of RSV to ICAM-1. A method of preventing RSV infection by administering an effective amount of an agent that down regulates the expression of ICAM-1, thereby decreasing RSV binding to ICAM-1 is also provided. There is provided a method of treating RSV infection by administering an effective amount of an agent for down regulating ICAM-1 expression. A method of blocking RSV-ICAM-1 interaction by administering an effective amount of agents for blocking ICAM sites of binding is provided. Also provided is a compound for blocking RSV-ICAM-1 interaction including an agent for blocking ICAM sites of binding.

DESCRIPTION OF THE DRAWINGS

Other advantages of the present invention will be readily appreciated as

the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

Figure 1 is a photograph showing the topography of ICAM-1 expression in RSV infected HEp2 cells by confocal microscopy;

Figures 2A-B are graphs showing the interaction of ICAM-1 with RSV; (A) shows RSV bound to and immobilized ICAM-1 in a dose dependent manner; (B) shows the inhibition of RSV binding to ICAM which is examined by pre-incubation of RSV with mAbs;

Figures 3A-C are photographs showing the anti-ICAM-1 mAb treatment and how it inhibits RSV infection in airway epithelial cells; and

Figure 4 A-B show the role of ICAM-1 in a murine model of RSV infection; the C57B1/6 wildtype and ICAM-1 KO mice were inoculated i.n. with RSV then after 4 days the mice were sacrificed and the lungs were taken out; RT-PCR was done to check the replication of RSV in the lung tissue of ICAM-1 KO and the wild type mice using the method set forth in the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Generally, the present invention provides a compound and method for protection and prevention against respiratory infection. More specifically, the method provides protection against RSV infection which can be accomplished by administering a pharmaceutically effective amount of an agent.

The agent can be in the form of antibodies to either ICAM-1 or RSV epitopes that interact with the other, antisense oligonucleotides for ICAM-1 or other agents that down regulate ICAM-1 expression, or agents that block RSV interaction with ICAM-1. Specifically, the agent should interfere with the binding of RSV to ICAM-1. By blocking the ICAM sites of binding there is provided protection from RSV infection. Alternatively, the compound of the present invention can be used for the treatment of an RSV infection. In the preferred

embodiment, the agent of the present invention blocks the RSV-F binding site on ICAM-1. This has been proven to be the most effective method of prevention and treatment.

The method of the present invention involves administering an effective amount of an agent to a patient sufficient to either prevent the RSV infection or treat the RSV infection. Applicable methods are known to those of skill in the art. Preferably, the agent will be administered to the airway epithelial cells either intranasally or orally. Other methods of administration, as are known to those of skill in the art can be used without departing from the present invention, such as injection or other means of inhalation. Specifically, the present invention provides a method of blocking RSV-ICAM-1 interaction thereby providing protection against the RSV infection by administering an agent with blocking capabilities to a patient in need of such treatment.

Intercellular adhesion molecule-1 (ICAM-1), which is expressed on nasal epithelial cells, is a receptor for rhinovirus and is shown by the inventors to play a role in RSV infection. The role of ICAM-1 in RSV infection was examined using *in vitro* and *in vivo* models of RSV infection. Conofocal microscopy indicates that RSV is co-localized with membrane ICAM-1 on cultured, RSV-infected HEp-2 human bronchial epithelial cells.

RSV is also shown by inventors to bind to immobilized ICAM-1. The antibody to RSV-F protein inhibits this binding by 80%, whereas an antibody to RSV-G protein only inhibits by 36%. In a mammalian two-hybrid assay, the RSV-F but not RSV-G gene product interacts with ICAM-1 in transfected NIH3T3 cells. Furthermore, preincubation of HEp-2 cells with a neutralizing monoclonal antibody to ICAM-1 inhibits RSV infection of these cells in a dose dependent manner. This antibody also inhibits RSV infection of primary normal human bronchial epithelial cells and transformed epithelial (A549) and macrophage (U937) cell lines. Moreover, mice deficient in ICAM-1 or treated with antibodies and anti-sense oligonucleotides for ICAM-1 are significantly ($\rho<0.01$) protected against RSV infection. Thus antibodies to RSV, ICAM-1 and ICAM-1 anti-sense oligonucleotides inhibit RSV-ICAM-1 binding and prevents infection.

The compounds of the present invention are administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, patient age, sex, body weight and other factors known to medical practitioners. The pharmaceutically "effective amount" for purposes herein is thus determined by such considerations as are known in the art. The amount must be effective to achieve improvement including but not limited to improved survival rate or more rapid recovery, or improvement or elimination of symptoms and other indicators as are selected as appropriate measures by those skilled in the art. Additionally, when the compound is being administered orally, there must be included a compound for preventing the degradation of the agent.

The agent is administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, and other factors known to medical practitioners. The "effective amount" for purposes herein is thus determined by such considerations as are known in the art.

A gene therapy according to the present invention is administered to the airways, e.g. nose, sinus, throat and lung, for example, as nose drops, by nebulization, vaporization or other methods known in the art.

In another embodiment of the present invention, the treatment includes administering to the patient an effective amount of a composition containing a recombinant construct comprising a nucleic acid sequence encoding the agent, the nucleic acid sequence being operatively linked to one or more transcription control sequences. Further, the nucleic acid sequence is expressed at or adjacent to respiratory epithelial cells and the agent results in reduced proliferation of the respiratory infection.

Another embodiment of the present invention relates to a method of protecting a host against respiratory infection by administering to the host an effective amount of a vector containing a construct having a nucleotide

sequence encoding the agent with a promoter sequence operatively attached thereto. Further, this construct contains a noninfectious, nonintegrating DNA sequence which controls the expression of the agent. Additionally, administration of the agent is in an amount sufficient to increase levels of the agent in the respiratory tract thus providing a protective response.

The above discussion provides a factual basis for the use of the compound and method for prevention of respiratory viral infection. The methods used with a utility of the present invention can be shown by the following non-limiting examples and accompanying figures.

EXAMPLES

GENERAL METHODS:

Gene therapy:

By gene therapy as used herein refers to the transfer of genetic material (e.g DNA or RNA) of interest into a host with subsequent expression to treat or prevent a disease. The genetic material of interest encodes a product, more specifically it encodes an anti-sense molecule that binds to mucleic acids encoding ICAM-1, thereby preventing its translation. For a review see, in general, the text "Gene Therapy" (Advances in Pharmacology 40, Academic Press, 1997).

According to the method of the present invention, a vehicle/method (transfection, transduction, homologous recombination, etc.) and an expression system as needed and then the modified cells are expanded in culture and returned to the host/patient. These genetically reimplanted cells have been shown to express the transfected genetic material

In *in vivo* gene therapy, the genetic material to be transferred is introduced into the cells of the recipient organism *in situ*, that is within the recipient. These genetically altered cells have been shown to express the transfected genetic material *in situ*.

The gene expression vehicle is capable of delivery/transfer of heterologous nucleic acid into a host cell. The expression vehicle may include elements to control targeting, expression and transcription of the nucleic acid in a cell selective manner as is known in the art. It should be noted that often the 5'UTR and/or 3'UTR of the gene may be replaced by the 5'UTR and/or 3'UTR of the expression vehicle.

The expression vehicle can include a promotor for controlling transcription of the heterologous material and can be either a constitutive or inducible promotor to allow selective transcription. Enhancers that may be required to obtain necessary transcription levels can optionally be included. Enhancers are generally any non-translated DNA sequence which works contiguously with the coding sequence (in cis) to change the basal transcription level dictated by the promoter. The expression vehicle can also include a selection gene as described herein below.

Vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989), Chang et al., *Somatic Gene Therapy*, CRC Press, Ann Arbor, MI (1995), Vega et al., *Gene Targeting*, CRC Press, Ann Arbor, MI (1995), *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston MA (1988) and Gilboa et al (1986) and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors. In addition, see United States patent 4,866,042 for vectors involving the central nervous system and also United States patents 5,464,764 and 5,487,992 for positive-negative selection methods.

Features that limit expression to particular cell types can also be included. Such features include, for example, promoter and regulatory elements that are specific for the desired cell type.

In addition, recombinant viral vectors are useful for *in vivo* expression of a desired nucleic acid because they offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. This is in contrast to vertical-type of infection in which the infectious agent spreads only through daughter progeny. Viral vectors can also be produced that are unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

As described above, viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of viral vectors utilizes its natural specificity to specifically target predetermined cell types and thereby introduce a recombinant gene into the infected cell. The vector to be used in the methods of the invention will depend on desired cell type to be targeted and will be known to those skilled in the art.

Retroviral vectors can be constructed to function either as infectious particles or to undergo only a single initial round of infection. In the former case, the genome of the virus is modified so that it maintains all the necessary genes, regulatory sequences and packaging signals to synthesize new viral proteins and RNA. Once these molecules are synthesized, the host cell packages the RNA into new viral particles which are capable of undergoing further rounds of infection. The vector's genome is also engineered to encode and express the desired recombinant gene. In the case of non-infectious viral vectors, the vector genome is usually mutated to destroy the viral packaging signal that is required to encapsulate the RNA into viral particles. Without such a signal, any particles that are formed will not contain a genome and therefore cannot proceed through subsequent rounds of infection. The specific type of vector will depend upon the

intended application. The actual vectors are also known and readily available within the art or can be constructed by one skilled in the art using well-known methodology.

The compound of the present invention is administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, patient age, sex, body weight and other factors known to medical practitioners. The pharmaceutically "effective amount" for purposes herein is thus determined by such considerations as are known in the art. The amount must be effective to achieve improvement including but not limited to improved survival rate or more rapid recovery, or improvement or elimination of symptoms and other indicators as are selected as appropriate measures by those skilled in the art.

The recombinant vector can be administered in several ways. It should be noted that it can be administered as the compound or as pharmaceutically acceptable salt and can be administered alone or as an active ingredient in combination with pharmaceutically acceptable carriers, diluents, adjuvants and vehicles. Local administration can provide a quicker and more effective treatment. Administration can also be performed by, for intranasally orally, subcutaneously or parenterally including intravenous, intraarterial, intramuscular, intraperitoneally, and intranasal administration as well as intrathecal and infusion techniques. Implants of the compounds are also useful. The patient being treated is a warm-blooded animal and, in particular, mammals including man. The pharmaceutically acceptable carriers, diluents, adjuvants and vehicles as well as implant carriers generally refer to inert, non-toxic solid or liquid fillers, diluents or encapsulating material not reacting with the active ingredients of the invention.

It is noted that humans are treated generally longer than the mice or other experimental animals exemplified herein which treatment has a length proportional to the length of the disease process and drug effectiveness. The doses may be single doses or multiple doses over a period of several days, but single doses are preferred.

The doses may be single doses or multiple doses over a period of several days. The treatment generally has a length proportional to the length of the disease process and drug effectiveness and the patient species being treated.

When administering the compound of the present invention parenterally, it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion). The pharmaceutical formulations suitable for injection include sterile aqueous solutions or dispersions and sterile powders for reconstitution into sterile injectable solutions or dispersions. The carrier can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils.

Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Nonaqueous vehicles such a cottonseed oil, sesame oil, olive oil, soybean oil, corn oil, sunflower oil, or peanut oil and esters, such as isopropyl myristate, may also be used as solvent systems for compound compositions. Additionally, various additives which enhance the stability, sterility, and isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. In many cases, it will be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the present invention, however, any vehicle, diluent, or additive used would have to be compatible with the compounds.

Sterile injectable solutions can be prepared by incorporating the compounds utilized in practicing the present invention in the required amount of the appropriate solvent with various of the other ingredients, as desired.

A pharmacological formulation of the present invention can be administered to the patient in an injectable formulation containing any compatible carrier, such as various vehicle, adjuvants, additives, and diluents; or the compounds utilized in the present invention can be administered parenterally to the patient in the form of slow-release subcutaneous implants or targeted delivery systems such as monoclonal antibodies, vectored delivery, iontophoretic, polymer matrices, liposomes, and microspheres. Examples of delivery systems useful in the present invention include: 5,225,182; 5,169,383; 5,167,616; 4,959,217; 4,925,678; 4,487,603; 4,486,194; 4,447,233; 4,447,224; 4,439,196; and 4,475,196. Many other such implants, delivery systems, and modules are well known to those skilled in the art.

A pharmacological formulation of the compound utilized in the present invention can be administered orally to the patient. Conventional methods such as administering the compounds in tablets, suspensions, solutions, emulsions, capsules, powders, syrups and the like are usable. Known techniques which deliver it orally or intravenously and retain the biological activity are preferred.

In one embodiment, the compound of the present invention can be administered initially by intravenous injection to bring blood levels to a suitable level. The patient's levels are then maintained by an oral dosage form, although other forms of administration, dependent upon the patient's condition and as indicated above, can be used. The quantity to be administered will vary for the patient being treated and will vary from about 100 ng/kg of body weight to 100 mg/kg of body weight per day and preferably will be from 10 mg/kg to 10 mg/kg per day.

Local administration is advantageous because there is no dilution effect and, therefore, a smaller dose is required to achieve expression in a majority of the targeted cells. Additionally, local inoculation can alleviate the targeting requirement required with other forms of administration since a vector can be used that infects all cells in the inoculated area. If expression is desired in only a specific subset of cells within the inoculated area, then promoter and regulatory elements that are specific for the desired subset can be used to accomplish this

goal. Such non-targeting vectors can be, for example, viral vectors, viral genome, plasmids, phagemids and the like. Transfection vehicles such as liposomes can also be used to introduce the non-viral vectors described above into recipient cells within the inoculated area. Such transfection vehicles are known by one skilled within the art.

EXAMPLE 1:

Materials and Methods

Cell culture, virus and infection. Bronchial epithelial cells, HEp-2 (ATCC CCL-23), and RSV A2 strain (VR-1302) were obtained from the ATCC, Rockville, MD. HEp-2 cells were grown at 37°C with 5% CO_2 in a minimum essential medium with Hank's salt, supplemented with 5% fetal bovine serum (FBS). HEp-2 cells were infected with RSV at 5 x 10^5 pfu/ml and left for absorbtion for 2h at 37°C with 5% CO_2, after which the virus inoculum was replaced by complete medium. Cells were trypsinized and harvested at various hours post infection. RSV infection was detectable as early as 4 hours post infection by RT-PCR using primers for RSV-N gene (Hegele et al., 1994). Also, the RSV-infected cells could be enumerated by fluorescence microscopy using FITC-conjugated antibodies to RSV.

Confocal microscopy. RSV infected (5 x 10^5 pfu/ml) HEp-2 cells grown on coverslips for 24 hours were fixed in ethanol, blocked for 1 hour with 1% BSA in PBS, pH 7.4 and double stained with goat anti-RSV polyclonal Abs and mouse anti-human [cam-1 MaB (bba-4) Each at 4 μ g/ml (R & D Systems, Minneapolis, MN) for 1 hour at 37°C. The cells were subsequently incubated for 1 hour with secondary antibodies: rabbit anti-goat IgG-PE conjugate and sheep anti-mouse Igg-FITC conjugate, and were finally mounted in DAPI antifade (Oncor, Gaithersburg, MD). The cells were scanned using an Oncor digital confocal microscope at 525nm for FITC, at 580nm for PE, at 350 nm for DAPI for counter staining of DNA; all three images were merged to confirm the co-localization of ICAM-1 and RSV in the same cell.

Assay for RSV-ICAM-1 Interaction. Soluble CAM-1 protein (R & D Systems,

Minneapolis, MN) was coated onto high affinity ELISA plates (Costar, Cambridge, MA) at 4°C over night at 100 ng/well in carbonate bicarbonate buffer, pH 9.5. All other incubations were done for 2 hours at 37°C. Wells were blocked in 1% BSA in PBS, pH 7.4 and then incubated with different dilutions of RSV (from 2 X 10^5 to 4 X 19^6 pfu/ml). For competition, RSV was either pre-incubated or simultaneously incubated with either anti-RSV-G mAb or anti-RSV-F mAb (20 to 100 μg/ml). Wells were washed in BSA-Tween-20 (0.1%), pH 7.4 and sequentially incubated with goat anti-RSV polyclonal Ab at 90 μg/ml (Chemicon, Temecula, CA) and anti-goat IgG HEP conjugate (Sigma, St. Louis, MO). Wells were washed and developed with the TMB (substrate). Color development was stopped after 30 minutes by addition of 0.2 M sulfuric acid. The optical density was measured at 450 nm.

Flow cytometry. HEp-2 cells were infected with RSV, which had been preincubated with mAb to either F or G, or mouse IgG at 4°C overnight with gentle shaking. Cells were harvested 24 hours post infection, washed in old PBS, pH 7.4 and incubated with goat anti-RSV polyclonal Ab (Chemicon, Temecula, CA) at 1:100 dilution for 1 hour on ice. Cells were then washed with 1 ml of FACS buffer (PBS, pH 7.4: 0.5% FBS; 1 mM EDTA) and incubated with rabbit anti-goat IgG PE conjugate for 1 hour on ice. Cells were again washed in FACS buffer and analyzed for RSV infected cells on a FACScan instrument (Becton Dickinson, Mountain View, CA).

Mammalian two-hybrid assay. Full-length cDNAs encoding human [CAM-1, RSV-F and RSV-G were amplified from mRNA of RSV infected HEp-2 cells using gene specific primers, each of which included an appropriate restriction site for subsequent cloning [ICAM-1: Forward-5'CCT GGC GAA TTC CAG ACA TCT GTG TCC CCC TCA, Reverse - 5'GTG TGG ATC CAC TGC CAC CAA TAT; F gene: Forward- 5' CAA GAA TTC ATG GAG TTG CTA ATC CTC AAA CA, Reverse- 5' CTA TGT CGA CTT AGT TAC TAA ATG CAA TAT TAT TTA; and G gene: Forward- 5' AAT GAA TTC ATG TCC AAA AAC AAG GAC CAA CGC, Reverse- 5'GTT GTC GAC TAA CTA CTG GCG TGG TGT GTT]. The ICAM-1 cDNA was cloned in-frame with the activation domain (AD) derived from VP16 protein of herpes simplex virus in the vector pVP16 (Clontech, Palo Alto,

CA). The cDNA encoding F or G protein was amplified, and cloned in frame to the GAL4 DNA-binding domain (DBD) of the pM vector. The orientation and the reading frame of all these fusion constructs were verified to be correct by restriction endonuclease analysis. The pG5CAT vector was used to detect protein-protein interaction by expression of CAT enzyme. CAT activity was assayed using ELISA (Boehringer Mannheim, Indianapolis, IN) following the manufacturer's protocol. The vectors, pM, pVP16 and pG5CAT, were transfected into NIH 3T3 fibroblast cells to determine the basal level of CAT activity.

ICAM-1 blocking *in vitro*. HEp-2 cells (10^5 cells/ml) were treated with different concentrations (100 to 400 μg/ml) of anti-ICAM-1 mAb (BBA 4) (R & D Systems, Minneapolis, MN) or purified mouse IgG_1 antibodies (isotype control) (Sigma, St. Louis, MO) for 3 hours at 37°C and were subsequently infected with RSV at 5 X 10^5n pfu/ml. Cells were harvested 24 hours post-infection.

Reverse transcriptase PCR analysis. The total RNA was isolated from the harvested cells and tissue samples using Trizol (Life Tech., Gaithersburg, MD). Random primed cDNA was prepared using superscript II RNAse H- reverse transcriptase (Life Tech., Gaithersburg, MD. The first strand cDNA product (1 μl) was amplified using *Taq* polymerase (Life Tech., Gaithersburg, MD). Forward and reverse primers used are as follows: RSV-N forward: 5'-GCG ATG TCT AGG TTA GGA AGA A-3'; reverse: 5'-GCT ATG TCC TTG GGT AGT AAG CCT-3' (Vignola et al., 1993); ICAM-1 forward: 5'-ATG GCT CCC AGC AGC CCC-3'; reverse: 5'-CAC CTG GCA GCG TAG GGT-3' and β-actin forward: 5'-CGC GAG AAG ATG ACC CAG-3'; reverse: 5'-ATC ACG ATG CCA GTC GTA C-3'. ALL PCR reactions were denatured at 95°C for 1', annealed at 56°C for 1' and extended at 72°C for 1 minute for 40 cycles. All amplifications were RNA specific, as no bands were seen in the control (no template) PCR samples. The reaction products were separated on 1.5% agarose gels.

Immunofluroscence. RSV infected HEp-2 cells 24 hours post-infection were fixed in chilled acetone for 10 minutes and air-dried. Cells were stained for 30

minutes at 37°C with FITC-labeled anti-RSV mAbs (Chemicon, Temecula, CA) in a humid chamber. The unbound antibodies were removed by washing three times in PBS-Tween-20 (0.2%) buffer, pH 7.4. The slides were air dried again and mounted on Fluromount G (Fisher, Pittsburgh, PA) and observed under fluorescent microscope. RSV positive cells (green fluorescence) were counted randomly from 15 different spots and from two different slides for the same treatment group and the percent of infected cells were plotted against the concentration of ICAM-1 mAb.

Animals and Virus Infection. All animal experiments were performed in accordance with protocols approved by the University of South Florida and James A. Haley Veteran's Hospital Animal Care Committee. Mice were obtained from Jackson Laboratory (Bar Harbor, ME) and maintained in pathogen free condition. Mice were infected with 50 μl RSV suspension (5 X 10^5 pfu/ml) under light anesthesia of Nembutal (Abbot Laboratories, North Chicago, IL) by intranasal administration. The pattern of RSV induced lung infection in mice was similar to the published reports (Anderson et al., 1990; Oppenshaw et al., 1995; Hsu et al., 1998).

ICAM-1 blocking *in vivo*. Mice were first intra-tracheally administered with the mixture of ICAM-1 pholphothioate anti-sense ODNs (300 μg/mouse) complexed with the cationic lipid, lipofectamine (2.5 μg ODN/μg lipofectamine), and goat anti-mouse ICAM-1 mAb (40 μg mouse). After 2 hours the same mice were incolulated intranasally with goat anti-mouse ICAM-1 mAb (50 μg mouse) and infected with RSV 2 hours post-treatment. Mice were sacrificed two days post-infection and lungs were collected to examine RSV replication in lung cells by RT-PCR.

Histopathology. Viral infection, immunohistopathology and scoring inflammatory lesions were performed as described (Tayler et al., 1984; Graham et al., 1988) with modifications. Mice were sacrificed on the fourth day post infection (pi) by overdose (0.6g/kg) of pentobarbital. Lungs were inflated via trachea, fixed in Zamboni's PAF fixative at 4°C for 24 hours and subsequently

transferred to 80% ethanol followed by paraffin embedding. The sections were stained with hematoxylin and eosin (HE). The slides were coded and scored in blind fashion twice each by three different individuals. The severity of lung pathology was scored on a scale of 0-3 indicating the degree of inflammation. The entire lung section was reviewed and pathological changes were evaluated for epithelial damage, peri-bronchovascular cell infiltrate and interstitial-alveolar cell infiltrate from both the ICAM-$1^{+/+}$ and ICAN-$1^{-/-}$ mice. Epithelial damage was scored as: 0 = no damage, 1 = increased cytoplasm of epithelial cells without desquamation, 2 = epithelial desquamation without bronchial exudate composed of inflammatory cells, 3 = bronchial exudate composed with desquamated epithelial cells and inflammatory cells. Peribronchovascular cell infiltrate was scored as: 0 = no infiltrate, 1 = infiltrate up to four cells, 2 = infiltrate five to ten cells, 3 = infiltrate more than ten cells. Interstitial-alveolar cell infiltrate was scored as: 0 = no infiltrate, 1 = mild, generalized increase in cell mass of the alveolar septa without thickening of the septa or significant airspace consolidation, 2 = dense septal infiltrate with thickening of septa, 3 = significant alveolar consolidation in addition to interstitial inflammation. Pathological scores were expressed as mean $\pm$ standard error of mean (SEM). Intraobserver variation was $< 5\%$.

Statistical analysis. The difference between the treated and control cells was analyzed by Student's t test. The percent infection between groups of mice was compared with chi-square test.

Pathological scores were expressed as mean $\pm$ standard error of mean (SEM) and statistical comparisons between two groups were made with Mann-Whittney Test. Differences between groups were considered significant as ρ values less than 0.05. All analyses were performed on a Macintosh Computer (Apple Computer Inc., Cupertino, CA) with Startview II software (Abacus Concepts, Berkely, CA).

Results and Discussion

Surface ICAM-1 expression on HEp-2 cells infected with RSV was assayed following RSV infection by flow cytometry. The uninfected HEp-2 cells showed constitutive expression (3.6%) of ICAM-1. Surface ICAM-1 expression increased by four and eight fold, respectively, 24 hours and 48 hours post RSV infection. RSV antigen expression was detected both on the plasma membrane and in the cytoplasm as determined by confocal microscopy, whereas ICAM-1 expression was localized mostly to the plasma membrane (Figure 1). The co-localization of RSV And ICAM-1 ON HEp-2 cell surfaces (Figure 1D) suggested that RSV directly binds to ICAM-1 on epithelial cells

More specifically, Figure 1 shows the topography of ICAM-1 expression in RSV-infected HEp-2 cells by confocal microscopy as described (Fixler, 1996). Figure 1A shows that RSV was distributed on the cell surface and in the cell cytoplasm (red), Figure 1B shows that ICAM-1 was localized mostly on the cell surface by FITC staining (green), and Figure 1C shows that the nuclei were visualized by staining with DAPI (blue). Figure 1D shows that superimposition of all the three images indicated the co-localization of RSV and ICAM-1 on the plasma membrane of RSV infected cells.

The direct binding of RSV to ICAM-1 was examined by ELISA. RSV bound to immobilized ICAM-1 in a dose-dependent manner (Figure 2A). The lack of ICAM-1 binding to conditioned medium in absence of RSV (control) indicated that the binding between ICAM-1 and RSV in specific (Figure 2A). *Pneumoviruses* encode two major surface glycoproteins, the G protein that presumably attaches to the cell surface receptor (Levine et al., 1987) and the F protein that mediates fusion of viral envelope to cell membrane (Hall et al., 1991). Antibodies to either G or F protein neutralize virus infectivity and seem to play a major role in protective immunity against RSV both in human and mice (Olmsted et al., 1986; Stott et al., 1987). To determine which of these RSV proteins interacts with ICAM-1, the RSV suspension was incubated with mAb to either F or G and the degree of inhibition of RSV binding to ICAM-1 was assayed. Incubation with mAb to either F or G decreased the RSV-ICAM-1 binding by 80% and 36%, respectively (Figure2B). These results show that RSV can bind to ICAM-1 in the absence of other cellular factors and that the RSV

binds to ICAM-1 primarily via its F protein.

The role of F vis-à-vis G protein on the binding of RSV to ICAM-1 on HEp-2 cells was investigated. RSV was incubated with mAb to F or G protein or mouse IgG_1 (control) and then used to infect HEp-2 cells and the percent of infected cells estimated by flow cytometry. Compared to control Abs, the mAb to F significantly inhibited (73.8%) the RSV infection of HEp-2 cells G protein, whereas the mAb to G did not. Furthermore, direct interaction of F or G protein with ICAM-1 protein was assayed by estimating chloramphenicol acetyl transferase (CAT) expression in a mammalian two-hybrid system. The transfection of NIH3T3 fibroblast cells with cDNA constructs expressing G, wherein ICAM-1 and CAT (pG5CAT) resulted in CAT activity similar to the basal level. In contrast, co-transfection of these cells with cDNA constructs expressing F, wherein ICAM-1 and CAT vectors resulted in a two-fold increase compared to basal CAT activity showing that interaction of RSV with ICAM-1 is mediated by F protein. Together, these results show that F protein binds to ICAM-1. The finding that the F and not G protein binds to ICAM-1 is consistent with the result noted and with other reports that antibodies to F protein have greater neutralizing ability than antibodies to G protein (Graham et al., 1988; Levine et al., 1987; Hall et al., 1991; Olmsted et al., 1986; Stott et al., 1987).

To explore the possibility that blocking of ICAM-1 would inhibit the initiation of RSV infection. HEp-2 cells were infected with RSV after having been incubated with various concentrations of a mAb to human ICAM-1. Since RSV infection begins when live RSV replicates in the host cell; the intracellular viral replication was determined 24 hours post infection using RT-PCR analysis for the expression of mRNA of the RSV nucleocapsid (N) gene. Preincubation of HEp-2 cells with anti-ICAM-1 inhibited amplifiable mRNA levels in a dose-dependent manner, with the greatest inhibition (70% compared to control) observed using a concentration of 400 μg of anti-ICAM-1 mAb/ml medium (Figure 3A). The reduction in virus replication was correlated with the RSV titers in the supernatant as determined by ELISA.

Inhibition of RSV infection was also determined by immunofluorescence

by scoring the RSV-infected cells stained with FITC-labeled mAb to RSV. Cells were counted randomly from fifteen different spots from two different slides of each group and the percentage of infected cells plotted against the concentration of anti-ICAM-1 mAb (Figure3B). A dose-dependent reduction in the number of infected cells occurred with increasing concentrations of anti-ICAM-1 mAb and the greatest inhibition of infection (96%) was observed at 400 μg/ml of anti-ICAM-1 mAb. In contrast, pre-incubation with the same concentration of isotype matched control antibodies showed no significant reduction in RSV replication. Two other cell lines, A549 (ATCC) and NHBE (Clonetics, San Diego, CA), were examined for inhibition of RSV infection by anti-ICAM-1 mAb, to exclude the inhibition an HEp-2 cell specific phenomenon NHBE is a normal human primary cell line derived from bronchial explants, whereas A549 is a transformed lung type II alveolar epithelial cell line. NHBE and A549 exhibited significant reductions (54% and 67%, respectively) in RSV infection when they were preincubated with 400 μg of anti-ICAM-1 mAb/ml (Figure 3B). These two cell lines differed from HEp-2 cells in the magnitude of RSV infection and inhibition of RSV infection. HEp-2 cells showed the greatest infection and inhibition of infection among the cell lines examined. The fact that preincubation of epithelial cells with anti-ICAM-1 mAb significantly inhibited both RSV replication and RSV infection indicates that ICAM-1 may be the principal molecule required for the initiation of RSV infection in these cells.

More specifically, Figure 3 shows anti-ICAM-1 mAb treatment inhibits RSV infection in airway epithelial cells. In Figure 3(A), preincubation of HEp-2 cells with anti-ICAM-1 mAb inhibited the expression of the RSV N gene. HEp-2 cells were treated either with a nti-ICAM-1 mAb 100 μg/ml (lane 4), 200 μg/ml (lane 5) or 400 μg/ml (lane 6), or mouse IgG_1 antibody (control) 400 μg/ml (lane 3), and subsequently infected with RSV (lanes 2-6 (Staunton et al., 1989). Total RNA was subjected to RT-PCR analysis using primers for the RSV N gene and β-actin (as internal control). RT-PCR products were quantified by densitometry, and the band intensity relative to β-actin was plotted (lower panel). In Figure 3(B), anti-ICAM-1 mAb treatment inhibited RSV infection of epithelial cells. The percent of RSV infected HEp-2, NHBE and A549 cells after pre-incubation with anti-ICAM-1 mAb or an isotype control Ab was estimated as described

(Tomassini et al., 1989). Each value represents mean ± SEM. The experiment was repeated for each cell line with similar results. A representative experiment for each of the cell lines is shown. Figure 3C shows inhibition of mRNA expression for RANTES and ET-1 by pretreatment of HEp-2 cells with anti-ICAM-1 mAb prior to infection. Total RNA was subjected to RT-PCR analysis using primers for RANTES, ET-1 and β-actin for each treatment (lanes 1-6 as described in A) was plotted.

To examine the *in vivo* role of ICAM-1 in the initiation of RSV infection, a murine model of RSV infection was adapted using C57B1/6 and BALB/c mice. Intranasal administration of RSV to C57BL6 and BALB/c mice induced lung infection, which peaked between day three and seven post infection. RSV replication was detectable in lung cells two to four days post infection, utilizing Rt-PCR, as described for HEp-2 cells. ICAM-1 deficient (ICAM-1^{-1-}) mice were used to evaluate the role of ICAM-1 in the initiation of RSV infection (Sligh et al., 1993). The RT-PCR analysis of RSV N gene mRNA demonstrated that in contrast to 52% (13/25) of C57BL6-ICAM-$1^{-/-}$, 88% (22/25) of the wild type (ICAM-$1^{+/+}$)mice permitted RSV replication in their lung tissue (Figure 4A). These results indicate a significant role of ICAM-1 in the initiation of RSV infection.

ICAM-1 antibodies and anti-sense oligodeoxynucleotides (ODNs) were previously found to protect mice from septic shock by decreasing pulmonary inflammation (Kumasaka et al., 1996) and the magnitude of protection ranged from 58 to 75% of control. However, the role of anti-ICAM-1 mAbs and ODNs against the initiation of RSV infection has not been previously examined. To confirm the *in vivo* role of ICAM-1 in RSV infection, BALB/c mice were intra-tracheally administered a combination of anti-ICAM-1 mAb and ICAM-1 anti-sense ODNs and subsequently infected with RSV. The RSV infection was monitored by RT-PCR of RSV-N gene mRNA from lung homogenate. The results demonstrate that pretreatment of BALB/c mice with a combination of ICAM-1 anti-sense ODNs and anti-ICAM-1 mAb decreased RSV N mRNA expression by 84% anti-ICAM-1 mAb decreased RSV N mRNA expression by 84% in RSV infected mice (Figure 4B). These results confirm that the presence

of ICAM-1 enhances the successful initiation of RSV infection *in vivo*.

More specifically, Figure 4 shows the analysis of RSV infection in mice. Figure 4(A) shows the RT-PCR analysis of the lung mRNA of RSV infected ICAM-$1^{+/+}$ and ICAM-$1^{-/-}$ C57BL6 mice (n=25 each) using RSV-N gene specific primers. Figure 4(B) shows the RT-PCR analysis of RSV infection in the lung RNA of BALB/c mice pre-treated with ICAM-1 anti-sense ODNs and anti-ICAM-1 mAb (lanes 1-4, PBS treated; and lanes 5-8, treated with mAb and ODNs) and subsequently infected with RSV (Oppenshaw 1995). Lung tissues were examined for replication by RT-PCR for RSV-N and β-actin (upper panel). RT-PCR products were quantified by densitometry, and the band intensity relative to β-actin for each mouse was plotted (Lower panel). Figure 4(C) shows RSV-induced lung pathology was evaluated for epithelial cell damage, interstitial-alveolar and peribronchovascular infiltrations (Vignola et al., 1993). The bars represent mean ± SEM; the asterics indicate the level significance at $\rho<0.05$; open circles, sham-infected ICAM-$1^{+/+}$; closed circles, sham-infected ICAM-$1^{-/-}$; open triangles, RSV-infected ICAM-$1^{+/+}$; closed triangles, RSV-infected ICAM-1.

Histopathology of the lung sections of the ICAM-$1^{-\backslash-}$ and ICAM-$1^{+\backslash+}$ mice [for each infected (n=13) and sham infected (n=8)] were analyzed to determine the pattern of inflammation induced by RSV. Pathological changes were determined by scoring for epithelial damage, interstitial-alveolar infiltrate and peribronchovascular infiltrates (Figure 4C). RSV infected ICAM-$1^{-\backslash-}$ mice exhibited significantly less ($\rho<0.05$) epithelial damage and interstitial-alveolar cellular infiltration compared to the ICAM-$1^{+/+}$ mice. No significant difference was seen in peri-bronchovascular infiltration between ICAM-$1^{-/-}$ and ICAM-$1^{+/+}$ mice. These results show that ICAM-1 deficiency provides a significant but only partial protection against inflammation due to RSV infection, similar to attenuated responses reported for neutrophil emigration in chemical peritonitis, in ischemic injury and septic shock in ICAM-$1^{-/-}$ mice (Sligh et al. 1993; Kelly et al., 1996; Xu et al., 1994). The protection in all of these disease models is believed to be primarily due to the role of ICAM-1 in mediating infiltration of inflammatory cells into the lung.

A significant finding from these studies is that ICAM-1 plays a pivotal role in the initial steps of RSV infection. RSV binds to ICAM-1 on the cell surface via its F protein. Blocking ICAM-1 with a neutralizing anti-ICAM-1 mAb or RSV with a mAb to F protein significantly inhibits RSV infection both in *in vitro* and *in vivo* models. Although these studies were confined to the RSV strain A_2, RSV are considered serologically monotypic, i.e., antiserum to A_2 neutralizes heterologous strains in *in vitro* assays and in experimental animals (Graham et al., 1988; Levine et al., 1987; Hall et al., 1991). The inventor's demonstration that a combination of anti-ICAM-1 mAb and ICAM-1 antisense ODNs prevented RSV infection of the lung cells in mice suggests that ICAM-1 mediation of RSV infection is not an epithelial cell specific phenomenon. Preincubation of a human macrophage cell line, U937, also inhibited RSV infection of these cells. Since RSV F-ICAM-1 binding is pivotal to RSV entry into the host cells, RSV can be neutralized using a soluble ICAM-1 may be potentially useful.

Throughout this application, various publications, including United States patents, are referenced by author and year and patents by number. Full citations for the publications are listed below. The disclosures of these publications and patents in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the described invention, the invention may be practiced otherwise than as specifically described.

REFERENCES

Burke and Olson, "Preparation of Clone Libraries in Yeast Artificial-Chromosome Vectors" in Methods in Enzymology, Vol. 194, "Guide to Yeast Genetics and Molecular Biology", eds. C. Guthrie and G. Fink, Academic Press, Inc., Chap. 17, pp. 251-270 (1991).

Capecchi, "Altering the genome by homologous recombination" Science 244:1288-1292 (1989).

Davies et al., "Targeted alterations in yeast artificial chromosomes for inter-species gene transfer", Nucleic Acids Research, Vol. 20, No. 11, pp. 2693-2698 (1992).

Dickinson et al., "High frequency gene targeting using insertional vectors", Human Molecular Genetics, Vol. 2, No. 8, pp. 1299-1302 (1993).

Duff and Lincoln, "Insertion of a pathogenic mutation into a yeast artificial chromosome containing the human APP gene and expression in ES cells", Research Advances in Alzheimer's Disease and Related Disorders, 1995.

Huxley et al., "The human HPRT gene on a yeast artificial chromosome is functional when transferred to mouse cells by cell fusion", Genomics, 9:742-750 (1991).

Jakobovits et al., "Germ-line transmission and expression of a human-derived yeast artificial chromosome", Nature, Vol. 362, pp. 255-261 (1993).

Lamb et al., "Introduction and expression of the 400 kilobase *precursor amyloid protein* gene in transgenic mice", Nature Genetics, Vol. 5, pp. 22-29 (1993).

Pearson and Choi, *Expression of the human b-amyloid precursor protein gene from a yeast artificial chromosome in transgenic mice.* Proc. Natl. Acad. Sci. USA, 1993. **90**:10578-82.

Rothstein, "Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast" in Methods in Enzymology, Vol. 194, "Guide to Yeast Genetics and Molecular Biology", eds. C. Guthrie and G. Fink, Academic Press, Inc., Chap. 19, pp. 281-301 (1991).

Schedl et al., "A yeast artificial chromosome covering the tyrosinase gene confers copy number-dependent expression in transgenic mice", Nature, Vol. 362, pp. 258-261 (1993).

Strauss et al., "Germ line transmission of a yeast artificial chromosome spanning the murine a_1 (I) collagen locus", Science, Vol. 259, pp. 1904-1907 (1993).

Gilboa, E, Eglitis, MA, Kantoff, PW, Anderson, WF: Transfer and expression of cloned genes using retroviral vectors. BioTechniques 4(6):504-512, 1986.

Cregg JM, Vedvick TS, Raschke WC: Recent Advances in the Expression of Foreign Genes in *Pichia pastoris*, Bio/Technology 11:905-910, 1993

Culver, 1998. Site-Directed recombination for repair of mutations in the human ADA gene. (Abstract) Antisense DNA & RNA based therapeutics, February, 1998, Coronado, CA.

Huston et al, 1991 "Protein engineering of single-chain Fv analogs and fusion proteins" in Methods in Enzymology (JJ Langone, ed.; Academic Press, New York, NY) 203:46-88.

Johnson and Bird, 1991 "Construction of single-chain Fvb derivatives of monoclonal antibodies and their production in *Escherichia coli* in Methods in Enzymology (JJ Langone, ed.; Academic Press, New York, NY) 203:88-99.

Mernaugh and Mernaugh, 1995 "An overview of phage-displayed recombinant antibodies" in Molecular Methods In Plant Pathology (RP Singh and US Singh, eds.; CRC Press Inc., Boca Raton, FL) pp. 359-365.

Collins, P.L. 1991. The molecular biology of human respiratory syncytial virus (RSV) of the genus Pneumovirus. In *The Paramyxoviruses*. D.W. Kingsbury, Editor, Plenum, New York, 1991. 103-162.

Murray, A.R., and S.F. Dowell. 1997. Respiratory syncytial virus: not just for kids. *Hospital Practice*, July 15:87-104.

Center for Disease Control and Prevention: Respiratory syncytial virus activity: United States. 1996-1997 season, *MMW* 45:1053, 1996.

Sly, P.D., and M.E. Hibbrt. 1989. Childhood asthma following hospitalization with acute viral bronchiolitis in infancy. *Pediatr. Pulmonol.* 7:153-158.

Jeng, M.J., and R.J. Lemen 1997. Respiratory syncytial virus and bronchiolitis. *Am. Fam. Physician.* 55: 1139-1146.

Armstrong, D.S., and S. Menahem. 1993. Cardiac arrythmias as manifestation of acquired heart disease in association with pediatric respiratory syncytial virus infection. *J. Ped. Child Health* 29:309-311.

Fixler, D.E. 1996. Respiratory syncytial virus infection in children with congenital heart disease. *Ped. Cardiol.* 17:163-168.

Persson, C.G., J.S. Erjefalt, M. Anderson, I. Erjefalt, L. Greiff, M. Korsgren, M. Linden, F. Sundler, and C. Svensson. 1997. Epithelium, microcirculation, and eosinophils-new aspects of the allergic airway *in vivo. Allergy* 52:241-255.

Becker, S., H.S. Koren, and D.C. Henke. 1993. Interleukin-8 expression in

normal nasal epithelium and its modulation by infection with respiratory syncytial virus and cytokines tumor necrosis factor, interleukin-1 and interleukin-6. *Am. J. Respir. Cell Mol. Biol.* 8:20-27.

Noah, T.L., and S. Becker. 1993. Respiratory syncytial virus-induced cytokine production by a human bronchial epithelial cell line. *Am. J. Physiol.* L472-L478.

Sabauste, M.C., D.B. Jacoby, S.M. Richards, and D. Proud. 1995. Infection of a human respiratory epithelial cell line with rhinovirus. Induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. *J. Clin. Invest.* 96:549-557.

Makgoba, M.W., M.E. Sanders, G.E. Ginther Luce, E.A. Gugel, M.L. Dustin, T.A. Springer, and S. Shaw. 1998. Functional evidence that intercellular adhesion molecule-1 (ICAM-1) is a ligand for LFA-1 dependent adhesion in T cell-mediated cytotoxicity. *Eur. J. Immunol.* 18:637-640.

Greve, J.M., G. Davis, A.M. Meyer, C.P. Forte, S.C. Yost, C.W. Marlor, M.E. Kamarck, and A. McClelland. 1989. The major human rhinovirus receptor is ICAM-1. *Cell* 56:839-847.

Staunton, D.E., J. Merluzzi, R. Rothlein, R. Barton, S.D. Marlin, and T.A. Springer. 1989. A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. *Cell* 56:849-853.

Tomassini, J.E., D. Graham, C.M. Dewitt, D.W. Lineberger, J.A. Rodkey, and R.J. Colonno. 1989. CDNA cloning reveals that the major group rhinovirus receptor on HeLa cells in intercellular adhesion molecule 1. *Proc. Natl. Acad. Sci. USA.* 86:4907-4911.

Chini, B.A., M.A. Fiedler, L. Milligan, T. Hopkins, and J.M. Stark. 1998. Essential roles of NF-kappa B and c/EBP in the regulation of intercellular adhesion molecule-1 after respiratory syncytial virus infection of human respiratory epithelial cell cultures. *J. Virol.* 72:1623-1626.

Stark, J.M., V. Godding, J.B. Sedgwick, and W.W. Busse. 1996. Respiratory syncytial virus infection enhances neutrophil and eosinophil adhesion to intercellular adhesion molecule-1. *J. Immunol.* 156:4774-4782.

Arnold, R., H. Werchau, and W. Konig. 1995. Expression of adhesion molecules (ICAM-1, LFA-3) on human epithelial cells (A549) after respiratory syncytial virus infection. *Int. Arch. Allergy Immunol.* 107:392-393.

Vignola, A.M., A.M. Campbell, P. Chanez, J. Bousquet, P. Paul-Lacoste, F.B. Michael, P. Godard. 1993. HLA-DR and ICAM-1 expression on bronchial epithelial cells in asthma and chronic bronchitis. *Am. Rev. Respir. Dis.* 148: 689-94.

Hegele, R.G., S. Hayashi, A.M. Bramley, and J.C. Hogg. 1994. Persistence of

respiratory syncytial virus genome and protein after acute bronchiolitis in guinea pigs. *Chest* 105: 1848-1854.

Anderson J.J., J. Nordan, D. Saunders, G.L. Toms, and R. Scott. 1990. Analysis of the local and systemic immune responses induced in BALB/c mice by experimental respiratory syncytial virus infection. *J. Gen. Virol.* 71, 1561-1570.

Oppenshaw, P.J.M. 1995. Immunity and immunopathology to respiratory syncytial virus: the mouse model. *Am. J. Res. Crit. Care Med.* 152, S59-62.

Hsu, S.C., D. Chargelegue, and M.W. Steward. 1998. Reduction of respiratory syncytial virus titer in the lungs of mice after intranasal immunization with a chimeric peptide consisting of a single CTL epitope linked to a fusion peptide. *Virology.* 240, 376-381.

Tayler, G., E.J. Scott, M. Hugh, and A.P. Collins. 1984. Respiratory syncytial virus infection in mice. *Infection and Immunity* 43:649-655.

Graham, B.S., M.D. Perkins, P.F. Wright, and D.T. Karzon. 1988. Primary respiratory syncytial virus infection in mice. *J. Med. Virol.* 26:153-162.

Levine, S., R. Klaiber-Franco, and P.R. Paradiso. 1987. Demonstration that glycoprotein G is the attachment protein of respiratory syncytial virus. *J. Gen. Virol.* 68:2521-2524.

Hall, C.B., E.E. Walsh, C.E. Long, and K.C. Schnabel. 1991. Immunity to and frequency of reinfection with respiratory syncytial virus. *J. Infec. Dis.* 163:693-698.

Olmsted, R.A., R.M. Chanock, and P.L. Collins. 1986. Expression of the F glycoprotein of respiratory syncytial virus by a recombinant vaccinia virus: comparison of the individual contributions of the F and G glycoproteins to host immunity. *Proc. Natl. Acad. Sci. USA* 83:7462-7466.

Stott, E.J., G. Taylor, L.A. Ball, K. Anderson, K.K. Young, A.M. King, and G.W. Wertz. 1987. Immune and histopathological responses in animals vaccinated with recombinant vaccinia viruses that express individual genes of human respiratory syncytial virus. *J. Virol.* 61:3855-3861.

Li, X., S. Sambhara, C.X. Li, M. Ewasyshyn, M. Parrington, J. Caterini, O. James, G. Gates, R.P. Du, and M. Klein. 1998. *J. Exp. Med.* 188:681-688.

Becker, S.W. Reed, F.W. Henderson, and T.L. Noah. 1997. RSV infection of human airway epithelial cells causes production of the beta-chemokine RANTES. *Am. J. Physiol.* 272 (Lung Cell. Mol. Physiol. 16): L512-L520.

Saito, T., R.W. Deskin, A. Casola, H. Haeberle, B. Olszewska, P.B. Ernst, R. Alam, P.L. Ogra, and R. Garofalo. 1997. Respiratory syncytial virus induces

selective production of the chemokine RANTES by upper airway epithelial cells. *J. Infect. Dis.* 175:497-504.

Behera, A.K., M. Kumar, H. Matsuse, R.F. Lockey, and S.S. Mohapatra. 1998. Respiratory syncytial virus induces the expression of 5-lipoxygenase and endothelin-1 in bronchial epithelial cells. *Biochem. Biophys. Res. Commun.* In pres.

Sligh, J.E., C.M. Ballantyne, and S.S. Rich, H.K. Hawkins, C.W. Smith, A. Bradley, and A.L. Beaudet. 1993. Inflammatory and immune responses are impaired in mice deficient in intercellular adhesion molecule-1. *Proc. Natl. Acad. Sci. USA.* 90:8529-8533.

Kelly, K.J., W.W. Williams Jr., R.B. Colvin, S.M. Meehan, T.A. Springer, J.C. Gutierrez-Ramos, and J.V. Bohventre. 1996. Intercellular adhesion molecule-1 deficient mice are protected against ischemic renal injury. *J. Clin. Invest.* 97:1056-1063.

Xu, H., J.A. Gonzalo, Y. St. Pierre, I.R. Williams, T.S. Kupper, R.S. Cotran, T.A. Springer, and J.C. Gutierrez-Ramos. 1994. Leukocytosis and resistance to septic shock in intercellular adhesion molecule-1 deficient mice. *J. Exp. Med.* 180:95-109.

Kumasaka, T., W.M. Quinlan, N.A. Doyle, T.P. Condon, J. Sligh, F. Takei, A.L. Beaudet, C.F. Bennett, and C.M. Doerschuk. 1996. Role of the intercellular adhesion molecule-1 (ICAM-1) in endotoxin-induced pneumonia evaluated using ICAM-1 antisense oligonucleotides, anti-ICAM-1 monoclonal antibodies, and ICAM-1 mutant mice. *J. Clin. Invest.* 97:2362-2369.

Almenar-queralt, A., A. Duperray, L.A. Miles, J. Felez, and D.C. Altieri. 1995. Apical topography and modulation of ICAM-1 expression on activated endothelium. *Am. J. Pathol.* 147:1278-1288.

Staunton, D.E., A. Gaur, P.Y. Chan, and T.A. Springer. 1992. Internalization of a major group human rhinovirus does not require cytoplasmic or transmembrane domains of ICAM-1. *J. Immunol.* 148:3271-3274.

Huguenel, E.D., D. Cohn, D.P. Dockum, J.M. Greve, M.A. Fournel, L. Hammond, R. Irwin, J. Mahoney, A. McClelland, E. Muchmore, A.C. Ohlin, and P. Scuderi. 1997. Prevention of rhinovirus infection in chimpanzees by soluble intercellular molecule-1. *Am. J. Respir. Crit. Care Med.* 155:1206-1210.

Bella, J., P.R. Kolatkar, C.W. Marlor, J.M. Greve, and M.G. Rossmann. 1998. The structure of the two amino-terminal domains of human ICAM-1 suggests how it functions as a rhinovirus receptor and as an LFA-1 integrin ligand. *Proc. Natl. Acad. Sci.* USA 95:4140-4145.

Casanovas, J.M., T. Stehle, J. Liu, J. Wang, and T.A. Springer, 1998. A dimeric crystal structure for the N-terminal two domains of intercellular adhesion molecule-1 *Proc. Natl. Acad. Sci. USA* 95:4134-4139.

5

CLAIMS

What is claimed is:

1. A method of preventing a respiratory infection by administering an effective amount of an agent for down-regulating ICAM-1 expression.

2. The method according to claim 1, wherein said administration step further includes administering the agent to airway epithelial cells.

3. The method according to claim 1, wherein said administration step further includes administering the agent intranasally.

4. The method according to claim 1, wherein said administration further includes administering the agent by inhalation.

5. The method according to claim 2, wherein said administration step further includes administering the agent orally.

6. The method according to claim 1, wherein said administration step includes injecting the agent.

7. A composition for the prevention of respiratory infection comprising an agent which regulates ICAM expression.

8. The composition according to claim 6, wherein said agent is selected from the group consisting essentially of antibodies to ICAM-1, antibodies to RSV epitopes, antisense oligonucleotides for ICAM-1, and agents which regulate ICAM-1 expression.

9. A method of preventing RSV infection by administering an effective amount of an agent that interferes with the binding of RSV to ICAM-1.

10. A method of preventing RSV infection by administering an effective amount of an agent that down regulates the expression of ICAM-1, thereby decreasing RSV binding to ICAM-1.

11. The method according to claim 10, wherein said administration step further includes administering the agent to airway epithelial cells.

12. A method of treating RSV infection by administering an effective amount of an agent for down regulating ICAM-1 expression.

13. A method of blocking RSV-ICAM-1 interaction by administering an effective amount of agents for blocking ICAM sites of binding.

14. The method according to claim 13, wherein said administering step further includes the step of blocking the RSV-F binding site.

15. A compound for blocking RSV-ICAM-1 interaction comprising an agent for blocking ICAM sites of binding.

16. The compound of claim 14, wherein said agent is selected from the group consisting essentially of antibodies to ICAM-1, antibodies to RSV epitopes, antisense oligonucleotides for ICAM-1, and agents which block ICAM sites of binding.

17. The compound according to claim 14, wherein said compound blocks the RSV-F binding site on ICAM-1.

18. The compound according to claim 16, wherein said compound blocks ICAM via the ICAM-1 anti-sense oligonucleotides.

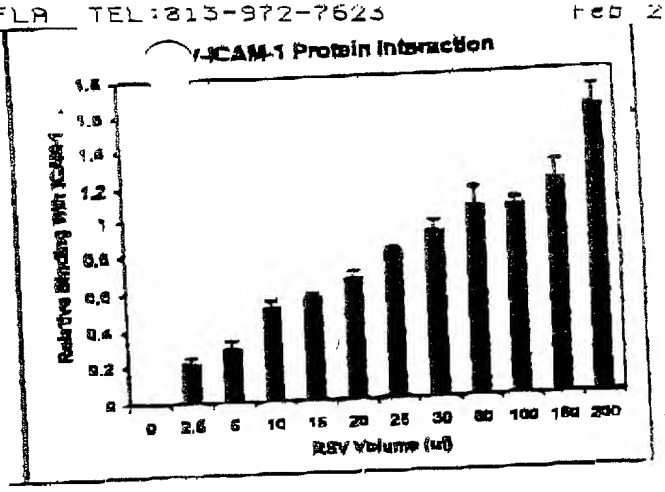
0152.00355

INTERRUPTING THE INTERACTION OF INTERCELLULAR ADHESION MOLECULE-1 AND RESPIRATORY SYNCYTIAL VIRUS TO FOR PREVENTION AND TREATMENT OF INFECTION

ABSTRACT OF THE DISCLOSURE

There is provided a method of preventing a respiratory infection by administering an effective amount of an agent for regulating ICAM-1 expression. Also provided is a composition for the prevention of respiratory infection including an agent which regulates ICAM expression. method of preventing RSV infection by administering an effective amount of an agent that interferes with the binding of RSV to ICAM-1. A method of preventing RSV infection by administering an effective amount of an agent that down regulates the expression of ICAM-1, thereby decreasing RSV binding to ICAM-1 is also provided. There is provided a method of treating RSV infection by administering an effective amount of an agent for down regulating ICAM-1 expression. A method of blocking RSV-ICAM-1 interaction by administering an effective amount of agents for blocking ICAM sites of binding is provided. Also provided is a compound for blocking RSV-ICAM-1 interaction including an agent for blocking ICAM sites of binding.

FIG 1



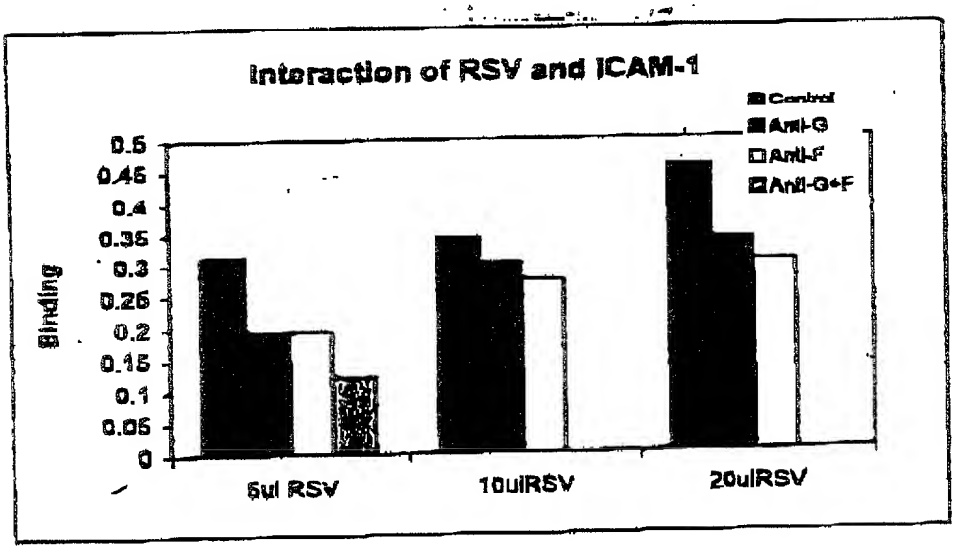


FIG 2

Example 5. Demonstration of direct interaction between RSV and ICAM-1 using ELISA. (A) The wells were coated with soluble ICAM-1 and incubated with increasing concentrations of RSV. After washing, the wells were incubated with an anti-RSV antibody. After washing of the wells, they were incubated with the corresponding second antibodies conjugated with alkaline phosphatase. The ELISA reactions were developed, read by ELISA reader and plotted. Results showed that increasing concentrations of RSV exhibited increasing OD values (binding). (B) The and RSV-G pr wells were coated with soluble ICAM-1 were incubated with increasing concentrations of RSV (5, 15 and 25 ul). The RSV was then incubated with monoclonal antibodies to RSV-F otein and with combination of antibodies to both F and G proteins for 1 hour. The interaction product was then incubated with ICAM-1 as in 'A'. After washing of the wells, they were incubated with the corresponding second antibodies conjugated with alkaline phosphatase. The ELISA reactions were developed, read by ELISA reader and plotted. The results showed that RSV may interact with ICAM-1 by its G and/or F proteins.

(6)

A.

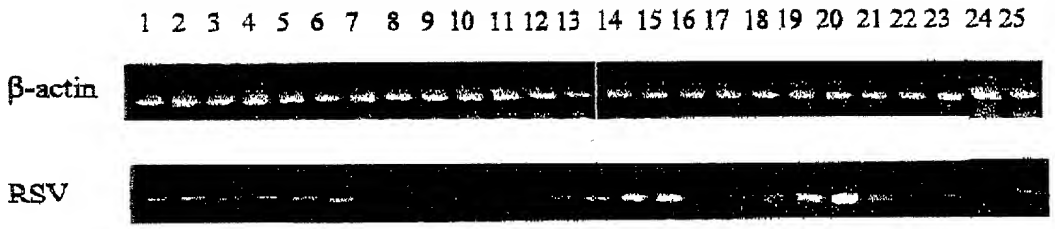
B.

C.

RSV	(-)	(+)	(+)	(+)	(+)
ICAM-1mAb	(-)	(-)	50 μg	100μg	200μg
M IgG Isotype	(-)	200μg	(-)	(-)	(-)

Fig 3

Wild type C57Bl/6



ICAM-1 deficient C57Bl/6

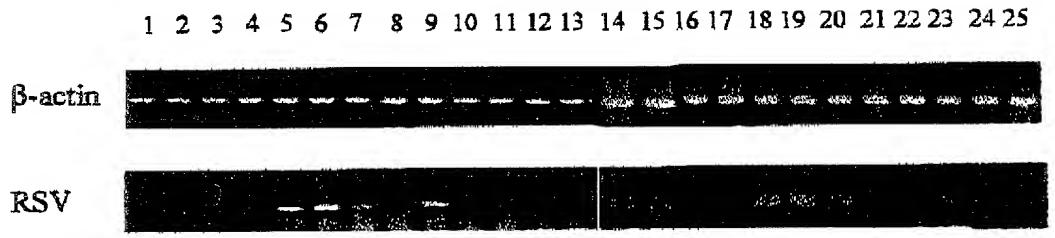


Fig 4.

Docket No.
0152.00355

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

INTERRUPTING THE INTERACTION OF INTERCELLULAR ADHESION MOLECULE-1 AND RESPIRATORY SYNCYTIAL VIRUS TO FOR PREVENTION AND TREATMENT OF INFECTION

the specification of which

(check one)

☒ is attached hereto.

☐ was filed on _____ as United States Application No. or PCT International Application Number _____

and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

60/123,999	March 11, 1999
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

Kenneth I. Kohn, Reg. No. 30,955
Amy E. Rinaldo, Reg. No. P-45,791

Send Correspondence to: **Kenneth I. Kohn**
KOHN & ASSOCIATES
30500 Northwestern Highway, Suite 410
Farmington Hills, Michigan 48334

Direct Telephone Calls to: *(name and telephone number)*
Amy E. Rinaldo - 248-539-5050

Full name of sole or first inventor **Aruna K. Behera**
Sole or first inventor's signature Date
Residence **4202 E. Fowler Avenue, FAO 126, Tampa, Florida 33620**
Citizenship **US**
Post Office Address **Same as above.**

Full name of second inventor, if any **Hiroto Matsuse**
Second inventor's signature Date
Residence **4202 E. Fowler Avenue, FAO 126, Tampa, Florida 33620**
Citizenship **US**
Post Office Address **Same as above.**

Full name of third inventor, if any
Mukesh Kumar

Third inventor's signature — Date

Residence
4202 E. Fowler Avenue, FAO 126, Tampa, Florida 33620

Citizenship
US

Post Office Address
Same as above.

Full name of fourth inventor, if any
Hamid Rabb

Fourth inventor's signature — Date

Residence
4202 E. Fowler Avenue, FAO 126, Tampa, Florida 33620

Citizenship
US

Post Office Address
Same as above.

Full name of fifth inventor, if any
Richard F. Lockey

Fifth inventor's signature — Date

Residence
4202 E. Fowler Avenue, FAO 126, Tampa, Florida 33620

Citizenship
US

Post Office Address
Same as above.

Full name of sixth inventor, if any
Shyam S. Mohapatra

Sixth inventor's signature — Date

Residence
4202 E. Fowler Avenue, FAO 126, Tampa, Florida 33620

Citizenship
US

Post Office Address
Same as above.